

10/031893

PCT/US00/19901

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Fig. 1

DRAP Complete cDNA Clone Insert

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agcgattacg gtagagatat ggtaatgcaa cggtggatgt gaactccttg      50
gtttgcggtg aatgcgttca acggtggatc ttcgcttgca cctttcgcg      100
tagacgacat acggatacag atacagatac agaatggcct ccaacaacag      150
tagtaccacc gatctggaca gccaggtcaa tgtggaggat ttgcccataa      200
cgttcaaggt gaagtacatt ggttccgaag tggcacgtgg cttatggggc      250
attaagtata cgcgtcgtcc ggttgacata atggtggggc tggccaagaa      300
cctgccgccc aataaggtgc tgcccaactg cgaactgaag gtgtccaccg      350
acggagtcga gctggagatc atatcgccaa aggccagcat caatcactgg      400
agctatccca tcgacacgat ctggtatggc gttcaggacc tgggtctacac      450
aagggctcttt gccatgatcg tgggtgaagga cgagtcgagt ccgcatccct      500
ttgaggttca cgccttcgtg tgcgacagtc gtgcgatggc gcggaagttg      550
acctttgccc tggccggccg ccttccagga ttactcgcg      600
aggcaaccgg tgaggaggag ggcgaggcca cgcccagcga cactattaca      650
cccacgogac acaagttcgc catcgatctg cgaacgcccg agaatccag      700
gctggcgaac tggagcagga aacggaggcg tagttatcct ggtgatcctg      750
cgttggtccc gtcaatgaga tgtgatgtgt tagttactta acgtccagt      800
ttcactgtat ctgtaaattg tggttctctc acctggtagt tgccctcaca      850
agctaattac ccaaagccta agtggttaata cgatttgtaa acgatttcta      900
aaataaatta cgaatatggt atgtttggct atttgaattg ggctacaacc      950
tggtgatatg ccacttgga aaaaaaaaaa acgccagcac caattctttt      1000
acttctgttt cttgtgaccg acataaaaga tgcaccaaag ctgctattcc      1050
accagcgttc tttattccac gcttgttttc atcattttgt cttccgtaag      1100
ataaattacg taaagcacca caggcatttt tatgtatttc tggagaatca      1150
taagatagca gtcgaactaa tgggtggata cctcccagag atcttgtagc      1200
ttgcttggtt ggatcatcca tgtagcaca atgctgtaga taggctgctg      1250
cattagcttt tatagcata ctcggttgcc ttaaaaagct tattacttct      1300
gaaagatttg gatcccga tctcattgta gaacaaatat cattttctga      1350
tccttcaatg taatcatcct tttcttcc      1378

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Longest ORF nt 104 - 610

Probable Start Codon nt 134

CDS Expressed as Recombinant Protein nt 134 - 610

Figure 2

With 206 enzymes: * MaxCuts: 1

B C M D
 a j m r
 e e e d
 I I I I

134 atggcctccaacaacagtagtaccaccgatctggacagccagggtcaatgtggaggatttg 193
 -----+-----+-----+-----+-----+-----+-----
 taccggagggtgtgtgtcatcatgggtggctagacctgtcgggtccagttacacctcctaacc

b M A S N N S S T T D L D S Q V N V E D L -

 P
 s
 P
 l
 4 DN rl B sP M HM
 0 da am w gs
 6 II Al o ae
 I IV II I II
 /

194 cccataacggttcaagggtgaagtacattggttccgaagtgccacgtggccttatggggcatt 253
 -----+-----+-----+-----+-----+-----+-----
 ggggtattgcaaggttccacttcatgtaaccaaggcttcaccgtgcaccgaataaccccgtaa

b P I T F K V K Y I G S E V A R G L W G I -

 B
 s
 t A
 1 f
 1 lM
 0 Il
 7 Iu
 I II
 /

254 aagtatacgctcggtccggttgacataatgggtggcggtggccaagaacctgccgcccaat 313
 -----+-----+-----+-----+-----+-----+-----
 ttcatatgcgcgagcgcccaactgtattaccaccgcaccgggttcttgagcgggcggtta

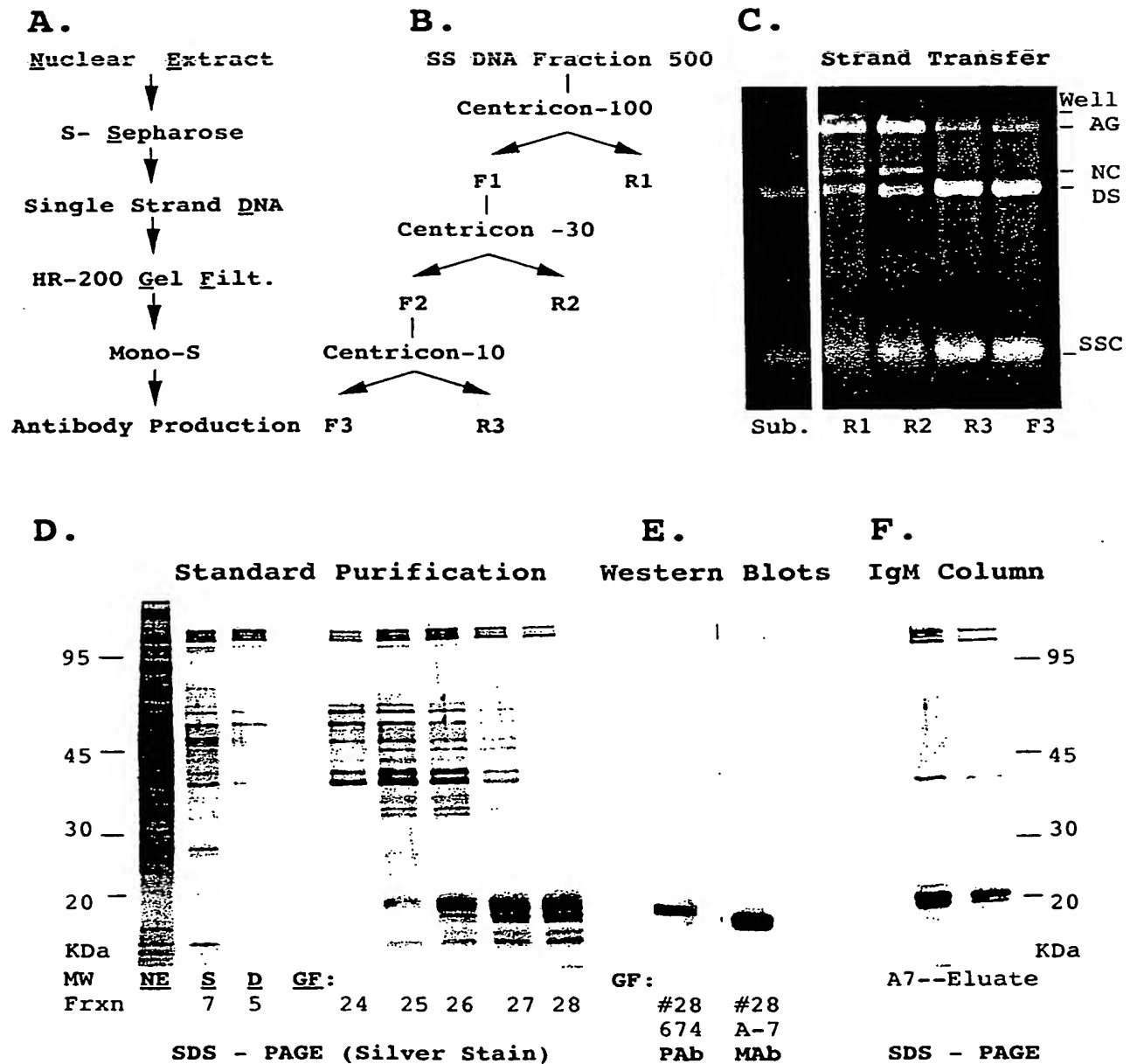
b K Y T R R P V D I M V G V A K N L P P N -

 E M
 c sP
 T o pv
 s 5 Au
 e 7 lI
 I I II
 /

314 aagtggtctgcccactgcgaactgaagggtgtccaccgacggagtccagctggagatcata 373
 -----+-----+-----+-----+-----+-----+-----
 ttccacgacgggttgacgcttgacttccacaggtgggtgcctcaggtcgacctctagtat

b K V L P N C E L K V S T D G V Q L E I I -

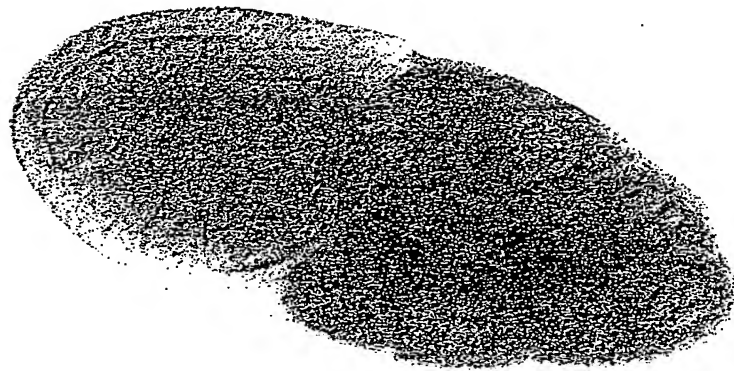
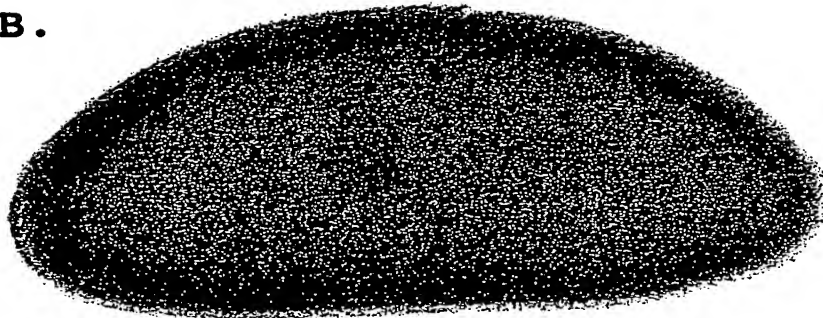
Figures 3A-3F

Recombination-Associated Protein Purification

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DRAP Transcript Distribution**A.****B.**

Figures 5A and 5B

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Figure 4 A-4D

Recombination-Associated Protein cDNA Clone

A. DRAP: 1378 bp Eco RI insert
ORF 104-610

1 134 281 902 970 1378
ATG ATG AATAAA (A)12 (A)29
#1 #50



B. → .. →

| | | | | | |
|------------|------------|------------|------------|------------|-----|
| MASNNSSSTD | LDSQVNVEDL | PITFKVKYIG | SEVARGLWGI | KYTRRPVDIM | 50 |
| VGVAKNLPPN | KVLPNCELKV | STDGVQLEII | SPKASINHWS | YIPDTISYGV | 100 |
| QDLVYTRVFA | MIVVKDESSP | HPFEVHAFVC | DSRAMARKLT | FALAGRLPGL | 150 |
| LATGGGGNR | | | | | 159 |

C.

| Potential | D1-----(30 to 70)---D2---(35+)---E1 | Motifs |
|-----------|--|--------|
| AA#s | (10 or 12)---(36 to 38)---48---(30)--- | 78 |
| | (19)---(54)---73---(44)--- | 117 |
| | (19 or 48)---(46 to 75)---94---(30)--- | 124 |

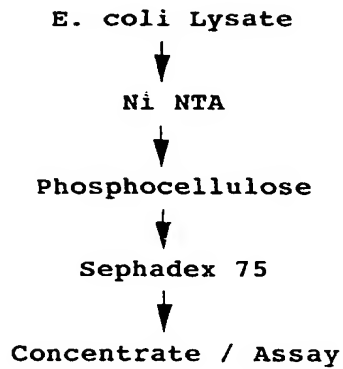
D.

| | | |
|-----------------------|---------------|-----------------------|
| Rad 51 | Mouse - Human | L L I V - D - S |
| Rad 51 | Yeast | L I V V - D - S |
| DCM 1 | Yeast | L I V V - D - S |
| RecA | E. Coli | V I V V - D - S |
| Drosophila DRAP | | M I V V K D E S S P |
| FLP Recombinase | | M I A L K D E T N P |
| T4 Gene 32 Protein | | I L V V K D P A A P |
| | | M I A V - D V E M G E |
| | | K - G F S S E |
| Human Topoisomerase I | | I K D E - - P |
| | | K D G S S E |
| | | G F S S P |

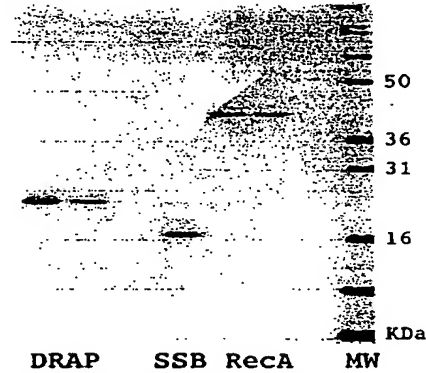
Figures 6A-6F

Recombination-Associated Protein Activities

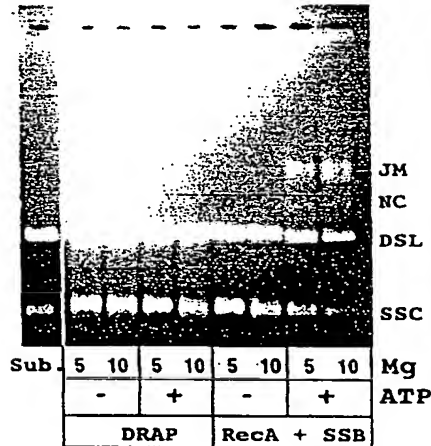
A.



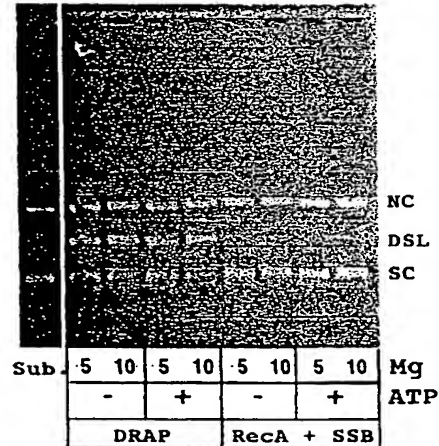
B.



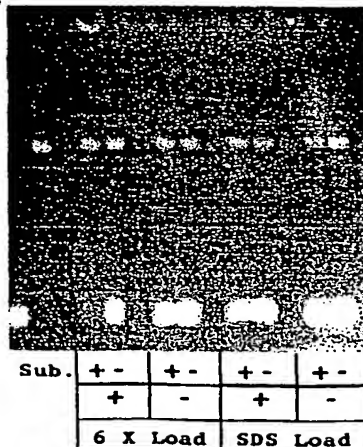
C.



D.



E.



F.

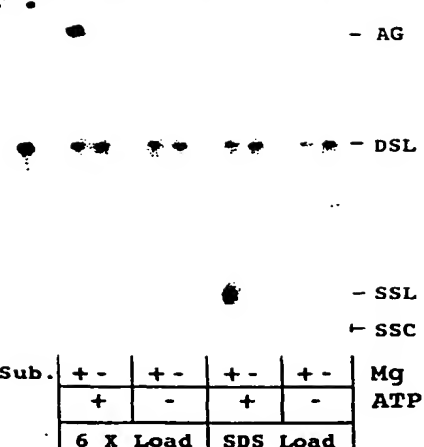


Figure 7

ATP-Dependent Three Strand Exchange Reaction

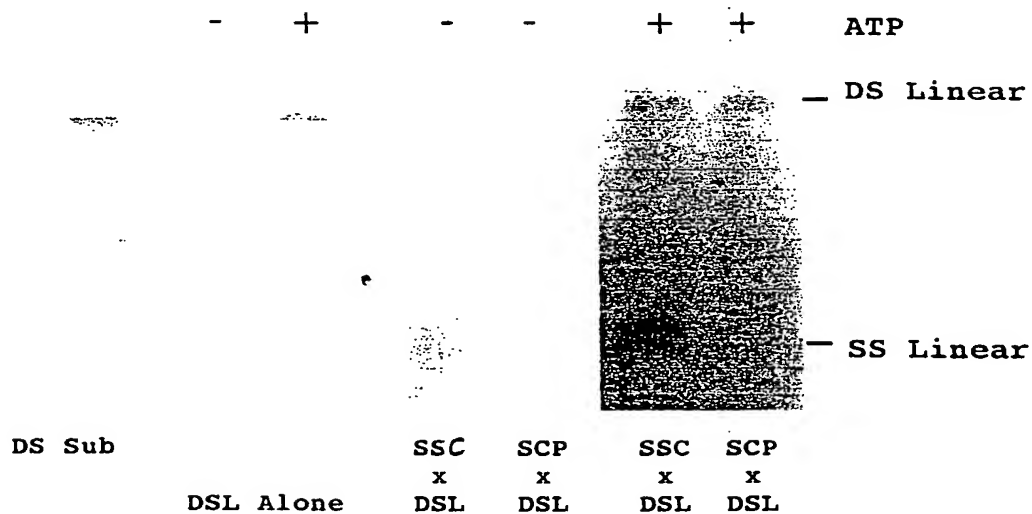


Figure 8

Nuclease Assay - 5' ^{32}P Oligo

(6% 1X TAE PAG)

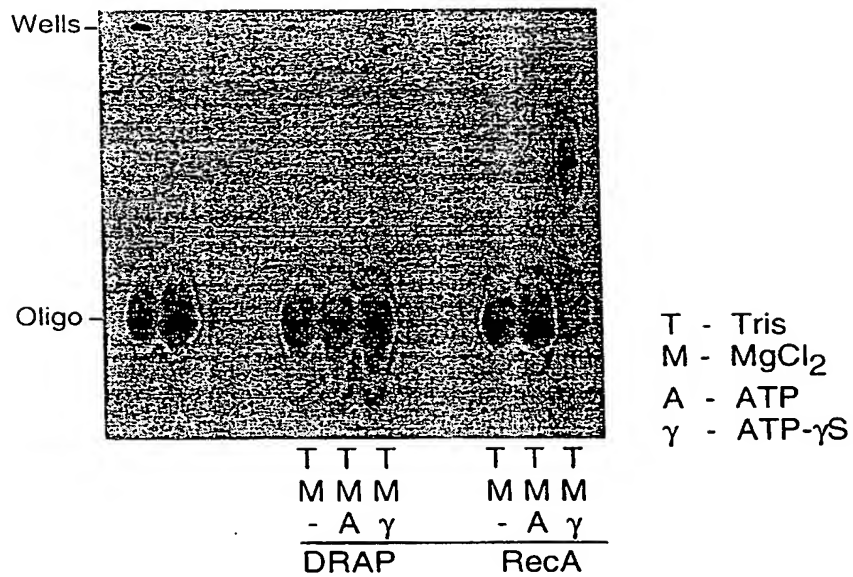
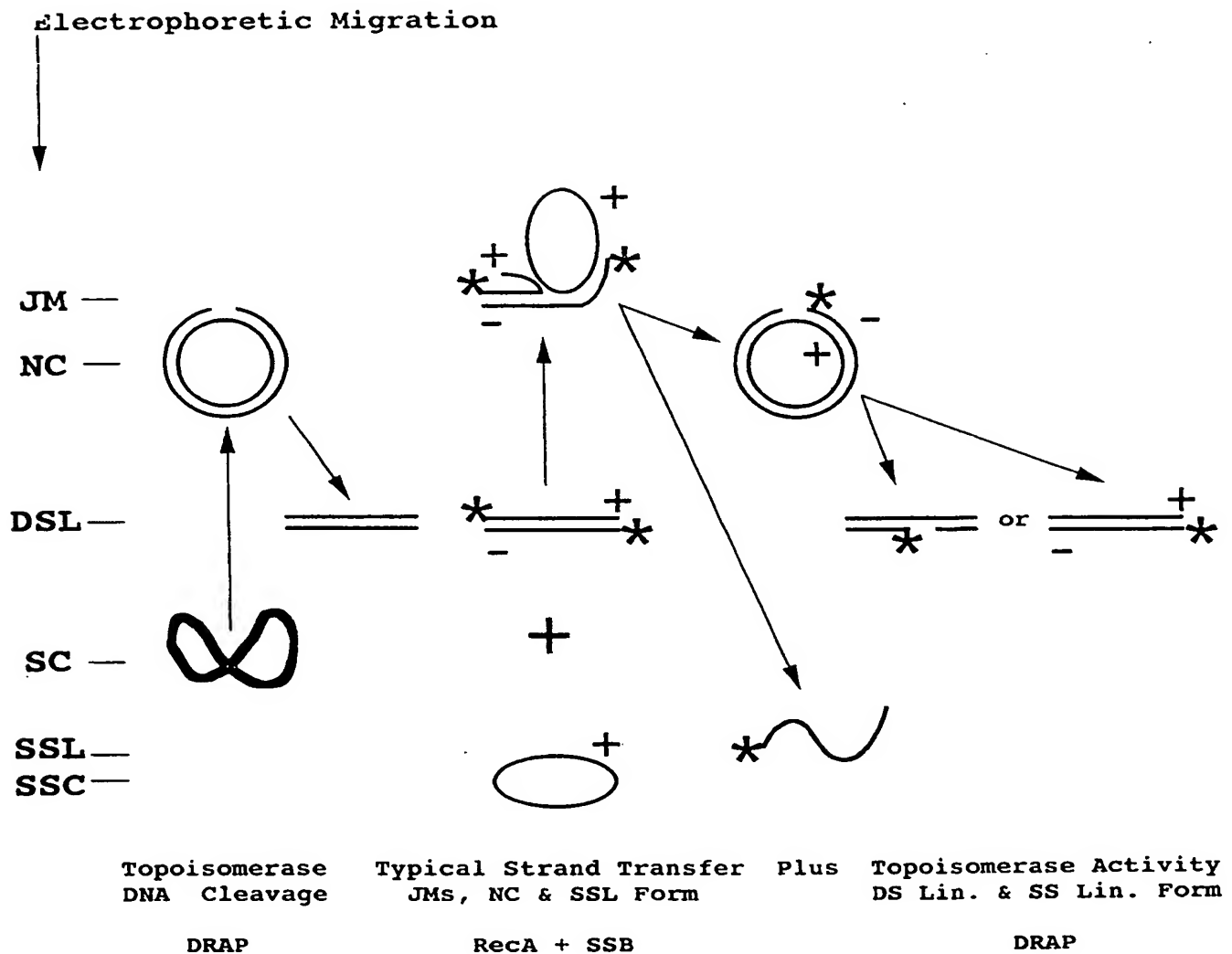
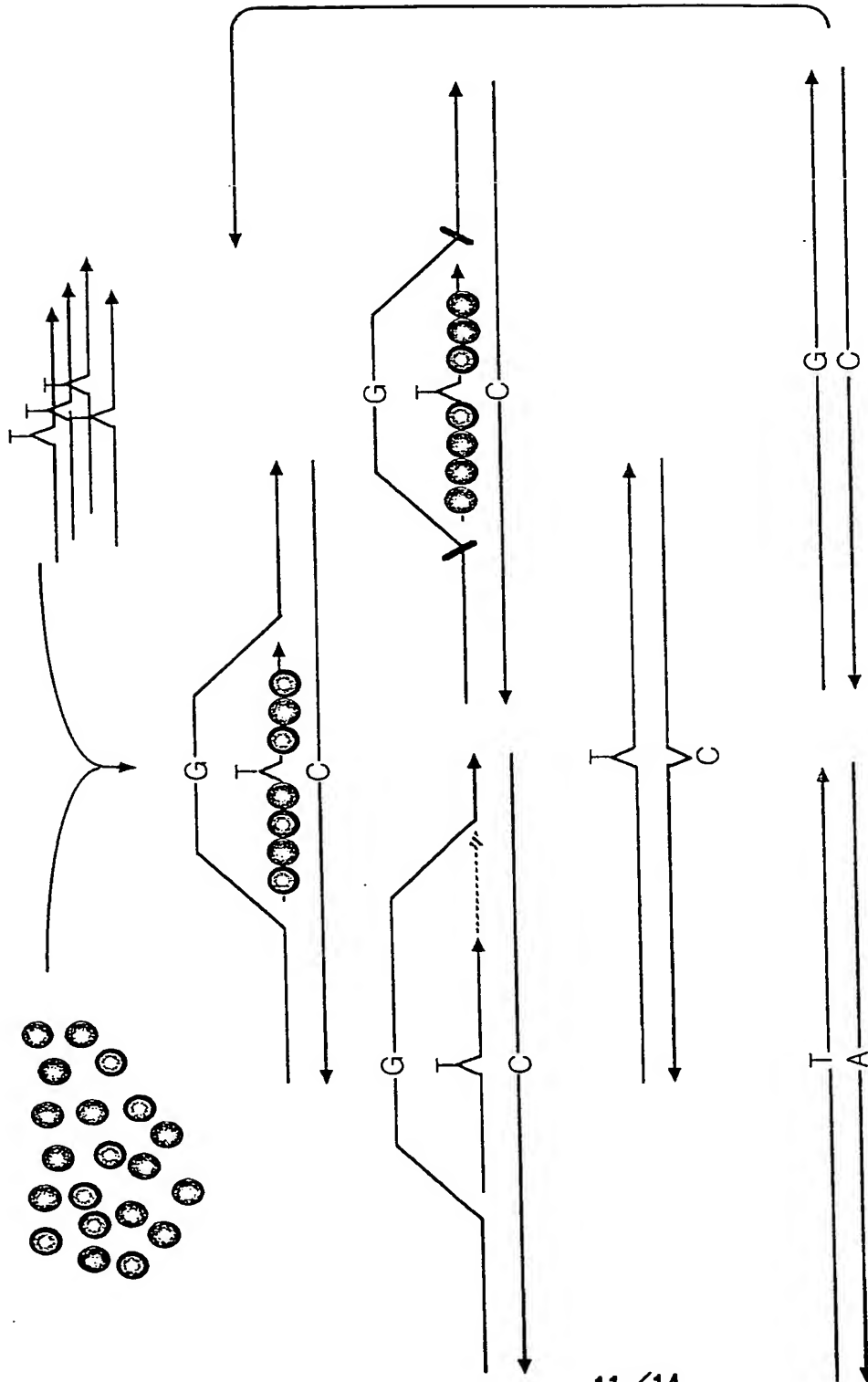


Figure 9

Strand Transfer and DNA Cleavage Reaction



Driving A Gene Conversion With A Recombinase and Oligos



Targeted Transgenics

| Gene | Oligo (No Phenotype) | Oligo + DRAP (Molar Ratio - Protein : Oligo) | |
|--------------------------------------|-------------------------|---|--|
| 1. N - myc (Exon 1) | 16 | Low (1:1) 60 OK High (100:1) 4 Alive 11 Stillborn | |
| 2. β 1 globin (Ala --> Val) | 7 | High (100:1) 10 OK 5 Runts 4 OK 1 Sickly | |
| 3. Agouti | 11 | High (100:1) 8 OK | |

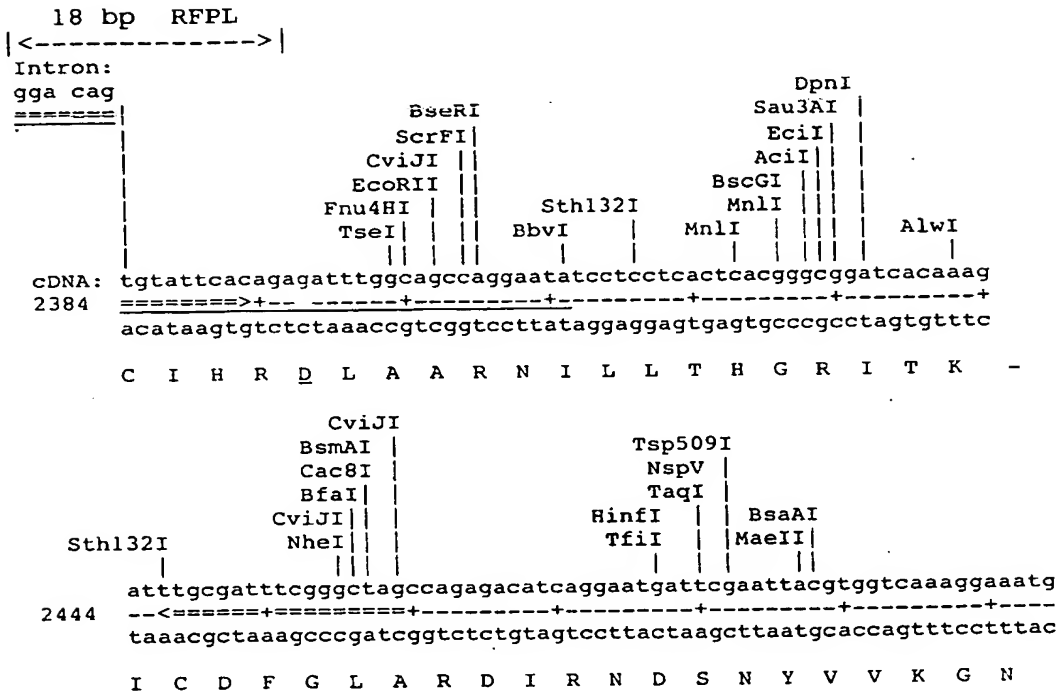
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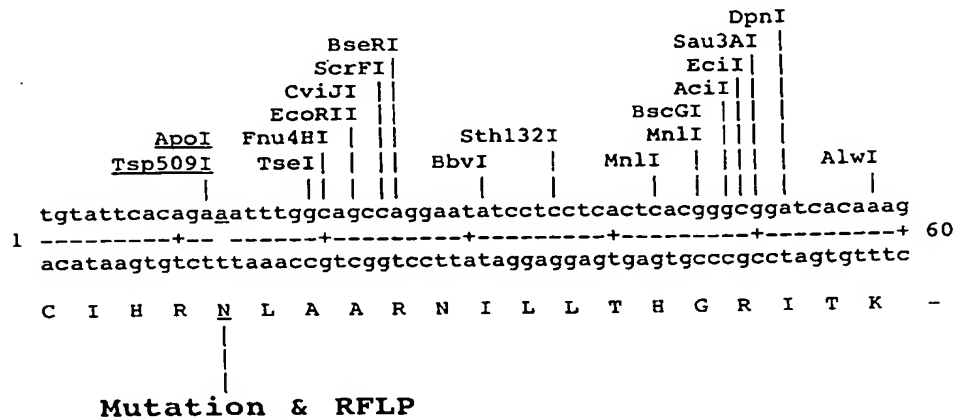
PCT/US00/19901

Figure 12

Murine c-Kit Exon 17



W42 Mutation



Underline:

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Potential Mutagenic Oligo

Potential PCR Primers for RFLP Analysis

Figure 13

Targeted Transgenic Mice



White-Spotted Mutant



Control Mouse